IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Application No. 10/531,448
Kazutomo Inoue
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Group Art Unit: 1651
Examiner: Macauley, Sheridan R

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks

Sir,

I, Yuanjun Gu declare that:

I was born in Shanghai, China, on December 23, 1961;

I am a citizen of China and a resident of Unit 302, No.38, Lane 8888, Zhongchun Road, Shanghai, 200063, China;

I graduated from Shanghai Second Medical University, Faculty of Medicine, Shanghai, China in 1985;

I have been a surgeon of Central Hospital of Xuhui District in Shanghai, China, since 1985 to 1987;

I have been a research student of Department of Surgery and Surgical Basic Science, Graduate School of Medicine, Kyoto University, Kyoto, Japan, since 1987 to 1997;

I received Ph.D. on the study of "Comparison of different collagenases in isolation of adult pig islets" from Kyoto University, Kyoto, Japan in 1997;

I have been a research associate of Institute for Frontier Medical Science, Kyoto University, Kyoto, Japan, since 1997 to

ATTACHMENT A

2003;

At present, I am a president of Jumpsun Bio-medicine (Shanghai) Co., Ltd from 2003 up to now;

I reported the following papers, for example;

1. Inoue K., Sumi S., Doi R. Yun M., Kaji H., Higashide S., Minote H., **Gu YJ.**, Kogire M., Hosotani R., Tobe T. Effect of VIP on splanchnic circulation in dogs. Biomedical Research, 9:125-129, 1988;
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5. Mitsuo M., Inoue K., Nakai I., Oda T., **Gu YJ.**, Shinohara S., Kogire M., Fujisato T., Maetani S., Ikada Y. Tobe T., Oka T. Efficacy of mesh reinforced polyvinylalcohol tube as a novel device for bioartificial pancreas; A functional study of rat islets in vivo. Transplantation Proceedings, 24:2939-2940, 1992;
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I., Hayashi H., Uchida K., Maetani S., Ikada Y., Tobe T. Isolation of adult pig islet; In vitro assessment and xenotransplantation. International Journal of Pancreatology, 12:173-180, 1992;

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9. Hosotani R., Doi R., **Gu Y.**, Wada M., Inoue K., Fujii N., Rayford P.L. Imamura M. Metabolism of cholecystokinin-33 in vivo: Effect of L-364,718, a CCK receptor antagonist. Annals of Clinical and Laboratory Science 24:346-354, 1994;
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11. **Gu YJ.**, Inoue K., Shinohara S., Doi R., Kogire M., Aung T., Sumi S., Imamura M., Fujisato T., Maetani S., Ikada Y. Xenotransplantation of bioartificial pancreas

using a mesh-reinforced polyvinyl alcohol bag. Cell Transplantation, 3:19-21, 1994;

12. **Gu YJ.**, Inoue K., Shinohara S., Doi R., Kaji H., Hayashi H., Aung T., Tun T., Echigo Y., Wada M., Imamura M., Iwata H. Comparison of different collagenases in isolation of adult pig islets. Cell Transplantation, 4:49-53, 1995;

13. Kogire M., Inoue K., Higashide S., Takaori K., Echigo Y., **Gu YJ.**, Sumi S., Uchida K., Imamura M. Protective effects of endothelin-1 on acute pancreatitis in rats. Digestive Disease & Sciences, 40:1207-1212, 1995;

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The experiment set out below was conducted under my supervision.

Experiment

1. Tested materials of the present invention

(Example 1) Preparation of a granule preparation containing freeze-drying fibrin

500 mg of fibrinogen (manufactured by Sigma) was gradually added to 500 ml of a PBS (-) solution (pH 7.2), and this was completely dissolved while stirring with a stirrer. 125 Units of thrombin (manufactured by Sigma) was added to the resulting fibrinogen solution, and this was stirred at room temperature for 1 hour. The precipitated fibrin was collected from a solution, and washed by stirring in 500 ml of distilled water for 30 minutes. Washing was repeated three times. After washing, a moisture of fibrin was removed using a filter (5A manufactured by ADVANTEC), and the fibrin was placed into a 50 ml centrifuge tube, and freeze-stored at -80°C overnight. Frozen fibrin was dried to obtain about 280 mg of granular fibrin. Freeze-drying was performed under conditions of temperature of -40°C and overnight using FDU-830 manufactured by Tokyo Rika Kikai.

Each 4 mg of the resulting granular fibrin was subdivided into Eppendorf tubes, gas-sterilized with a gas sterilizer (Ioject SA-360 manufactured by Nishimoto Sangyo Co., Ltd.), and stored at room temperature.

2. Experimental Method

[Example 2]

Nembutal (50 mg/kg) was intraperitoneally administered to a nude mouse (Japan SLC, Inc., BALB/C-nu), 8 to 10 week old, to anesthetize the animal, three sides of a skin on the median line part of the back were opened into a square having a traverse direction 1 cm and a length direction 2 cm without opening one side (base side: Base of Flap) in a traverse direction at a position 1 cm from an scapula, and this was peeled to prepare a skin flap(see Fig. 1).

Fibrin prepared in Example 1 was administered at 4 mg/one mouse. An administration method was performed by suspending 4 mg of fibrin in 20 μl of PBS (-) in an Eppendorf tube, and uniformly topically applying the solution between a skin flap and a subcutaneous tissue with a spatula. Immediately after the application, the opened part was sutured. This procedure was repeated to produce a model group (9 animals)(n=9) receiving the fibrin prepared in Example 1. As a control group, there were produced 9 mice (n=9) to which only 20 μl of PBS (-) without addition of the fibrin prepared in Example 1 had been administered. After suturing, the administration model group and the control group were returned to a rearing cage, and were reared by usually giving a solid feed and water.

[Example 3] Measurement test of blood flow amount

In an administration model group (n=1) and a control group (n=1) prepared in Example 2, blood flow at the central part of a skin flap on day 3 and on day 7 after skin flap formation=was investigated.

Mice of both groups were fixed on an experimental stand, a laser irradiating part of a laser Doppler apparatus (Model

ALF2100, manufactured by Advance Co., Ltd.) was put on the central part of a sutured skin flap surface at the back part, and change in blood flow amount was investigated for a constant time. Thereupon, measurement was performed by adhering an irradiation part and a mouse skin surface as much as possible. Fig. 2 (a), (b), (c) and (d) show change in blood flow amount at a time zone during which a stable blood flow amount was obtained. And, (a) and (b) show a blood flow amount (ml/100 g tissue/min) in both groups on day 3 after skin flap formation of, and (c) and (d) show a blood flow amount (ml/100 g tissue/min) in both groups on day 7 after skin flap formation.

From these results, the following was made clear. A blood flow amount of the administration model group on day 3 after skin flap formation was changed in about 14 to 16 ml/100 g tissue/min, and a blood flow amount of the control group was changed in about 4 to 5.5 ml/100 g tissue/min. In addition, on day 7 after skin flap formation, a blood flow amount of the administration model group was changed in about 11 to 20.5 ml/100 g tissue/min, and a blood flow amount of the control group was changed in about 4.5 to 5.5ml/100 g tissue/min, respectively. Therefore, in both of day 3 and day 7, a blood flow amount of the administration model group shows a more remarkably high value compared to the control group, and it was made clear that blood flow amount was elevated by administration of fibrin prepared in Example 1.

[Example 4] Blood flow amount recovery test

In an administration model group (n=5) and a control group (n=4) prepared in Example 2, the recovery rate of a blood flow

amount in a skin flap on day 1, day 3 and day 7 after skin flap formation was investigated.

Mice of both groups were fixed on an experimental stand, a line was provided in such a manner that a sutured skin flap surface on the back part (traverse direction 1 cm, length direction 2 cm square) was divided into 4 (in a length direction (length 2 cm is divided at 0.5 cm intervals), and divided into 3 in a traverse direction (traverse 1 cm is divided at about 0.33 cm intervals)). Four places of intersections of lines at a position 0.5 cm and a position 1.5 cm from a base side, and a line dividing into 3 in a traverse direction were marked, and a laser irradiating part of a laser Doppler apparatus (Model ALF2100, manufactured by Advance Co., Ltd.) was put on those four places to measure a blood flow amount (ml/100 g tissue/min). Thereupon, measurement was performed by adhering an irradiation part and a mouse skin surface as much as possible. An average of blood flow amounts at two points on a line at a position 0.5 cm from a base side was adopted as a blood flow amount at a 0.5 cm position, and an average of blood flow amounts at 2 points on a line at a position 0.5 cm from a base side was adopted as a blood flow amount at a 1.5 cm position. Letting an average of a blood flow amount obtained by measuring blood flow amounts at similar four places in advance to be 100, in mice of each of both groups before skin flap formation, a recovery rate of a blood flow amount was expressed as a ratio (%) relative to this 100. And, t-test was performed to obtain a significant difference between both groups.

Results of a blood flow recovery rate at a 0.5 cm position and a 1.5 cm position are shown in Fig. 5 (a) and (b), respectively.

In the control group, at a 0.5 cm position near a base side, recovery of blood flow amount was around 70.4 ± 13.29% (Mean ± SE) even on day 7 and, at a 1.5 cm position, only recovery of 5.23 ± 8.27% was obtained even on day 7. To the contrary, in the administration model group, a blood flow amount at a 0.5 cm position near a base side on day 3 was recovered to approximately 100% and, even at a 1.5 cm position, recovery of 81.75 ± 16.29% was seen on day 7. Therefore, it was made clear that, by administration of fibrin prepared in Example 1, remarkable recovery of blood flow amount is obtained.

[Example 5] Measurement test of Blood flow amount in rat ischemia model

Nembutal (50 mg/kg weight) was intraperitoneally administered to a rat (Shimizu Laboratory Supplies Co., Ltd., Kyoto), 8 to 10 week old, to anesthetize the animal. At an inner side of the rat right femoral groin, the femoral artery was completely cut to create an ischemic region at the right inferior limb (ischemia model). 8 mg of fibrin prepared in Example 1 was suspended sterile in 400 μl of PBS(-) in an Eppendorf tube, and each 100 μl of the solution was administered to four places of the right inferior limb ischemic region of an ischemia model by injection (administration model group, n=1). A blood flow amount of the light inferior limb ischemic region on day 5 after cutting of the femoral artery was measured using a laser Doppler apparatus (Model ALF2100, manufactured by Advance Co., Ltd.). A measurement method was according to Example 3, and blood flow amount in an ischemic region was measured. Only 400 μl of PBS (-) containing no fibrin was administered to a control group

(n=1).

3. Experimental Results

(1) Detailed experimental data of Example 3 and 4 are shown in Table 1.

Table 1:

		Day 3 after formation of skin flap		Day 7 after formation of skin flap	
		Measurement position			
		0.5 cm	1.5 cm	0.5 cm	1.5 cm
Control group	Blood flow amount	7.38 ± 1.0	1.62 ± 0.82	9.25 ± 0.73	0.62 ± 0.61
	Recovery rate (%)	56.1 ± 7.57	14.4 ± 7.4	70.4 ± 13.29	5.23 ± 8.27
Administration group	Blood flow amount	15.16 ± 1.5	8.53 ± 0.9	17.41 ± 1.36	10.2 ± 1.08
	Recovery rate (%)	100.2 ± 8.36	67.95 ± 14.7	117.83 ± 15.17	81.75 ± 16.29

Blood flow amount: ml /100g tissue /min

(2) Results of the administration model group and the control group of Example 5 are shown in Fig. 3 (a) and (b), respectively. The blood flow amount of the administration model group was changed in about 12 to 13 ml/100 g tissue/min, and blood flow amount of the control group was changed in about 4 to 4.5 ml/100 g tissue/min. The blood flow amount of the administration model group shows a remarkably higher value, compared to the control group, and it was made clear that, improvement in a blood flow amount is seen by administration of fibrin prepared in Example 1.

Fig. 1:

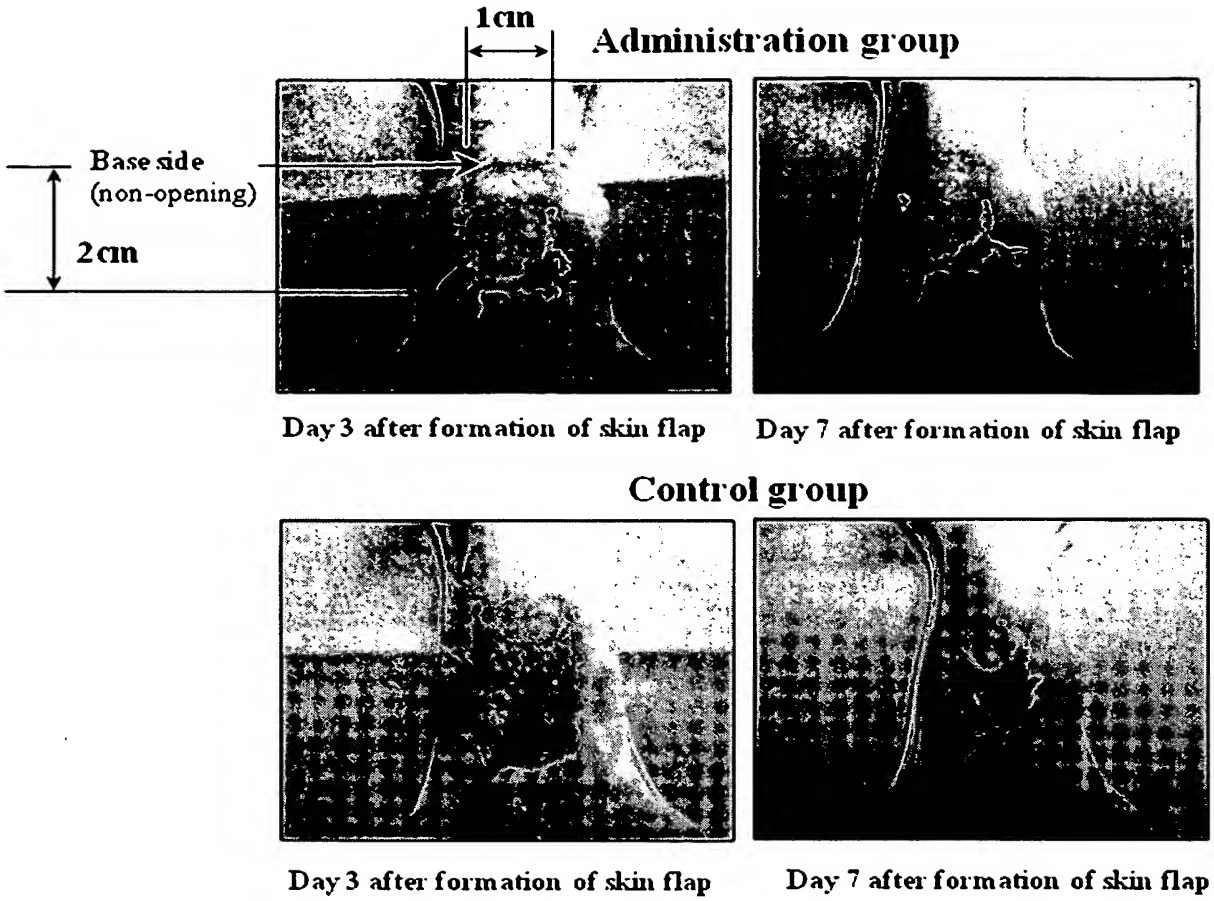

1cm
Administration group
Base side
(non-opening)
2cm
Day 3 after formation of skin flap
Day 7 after formation of skin flap
Control group
Day 3 after formation of skin flap
Day 7 after formation of skin flap

Fig. 2:

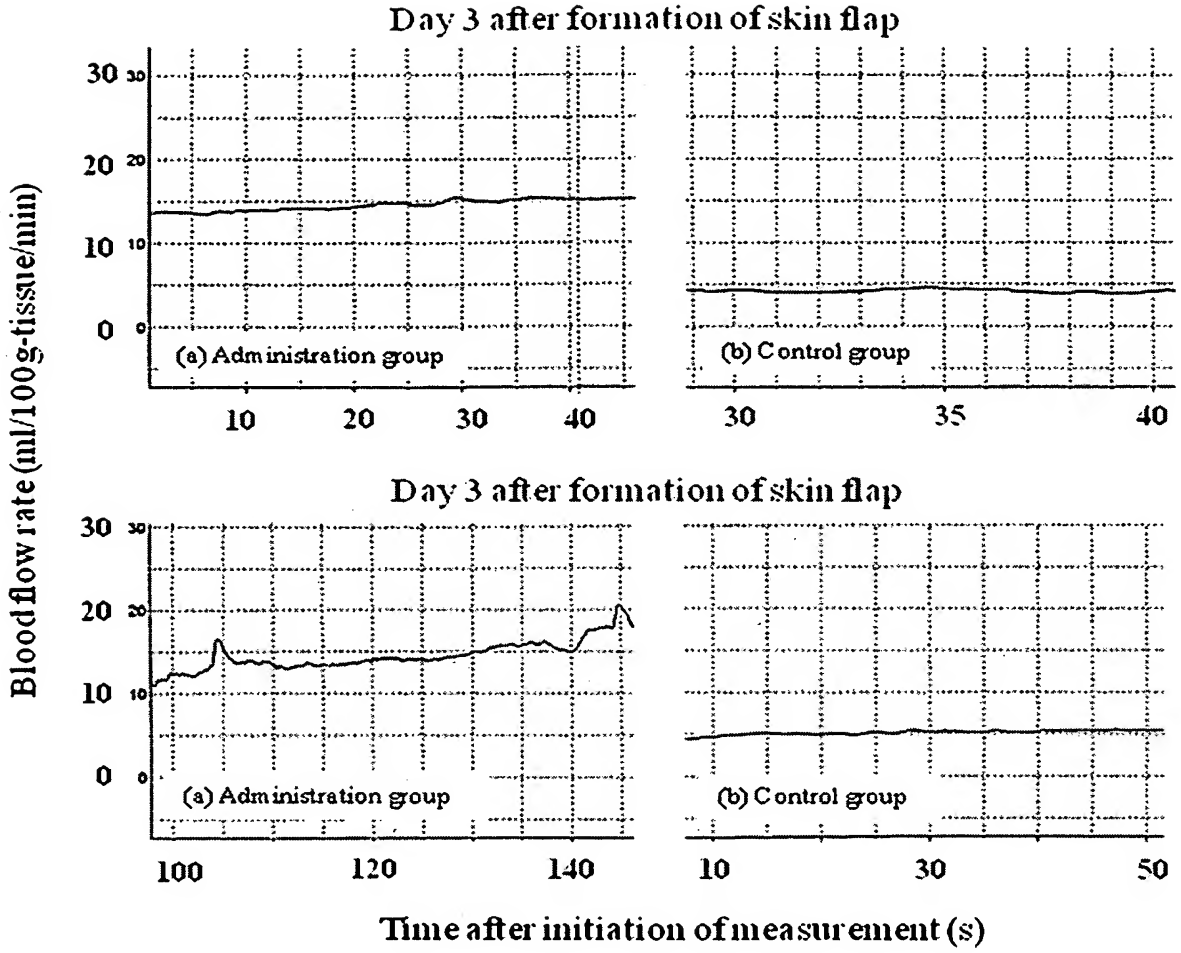
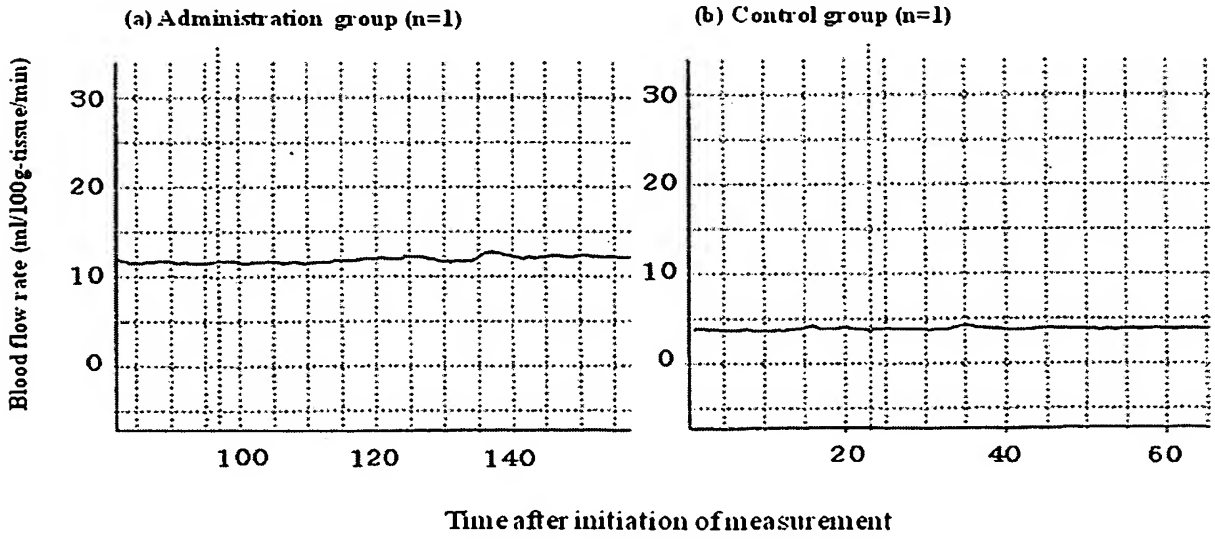

Day 3 after formation of skin flap
Blood flow rate (ml/100g-tissue/min)
(a) Administration group
(b) Control group
Day 3 after formation of skin flap
(a) Administration group
(b) Control group
Time after initiation of measurement (s)

Fig. 3:


(a) Administration group (n=1)
(b) Control group (n=1)
Blood flow rate (ml/100g-tissue/min)
Time after initiation of measurement

It is declared by the undersigned that all statements made herein of undersigned's own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 30th day of October, 2008

Yuanjun GU